TITLE TESTING ANTI-hPTK6-IgG FUSION – SERA

Proj No 9211
Book No.

From Page No.

Aim

Two rabbits have been immunised with the hptk6-IgG fusion protein purified by W Barson.

These sera will contain Ab's against both the human hptk6 and the IgG (F_c) domain.

– Need to test these sera

Ran 20µg of a) hptk6-IgG fusion protein

b) hptk5-IgG fusion protein

on 8% SDS – polyacrylamide gels

; Blotted to N/c filters

Ponceau stained filters after transfer &

prepared strips for testing.

– Photocopies of filters attached over.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	30th Nov 97

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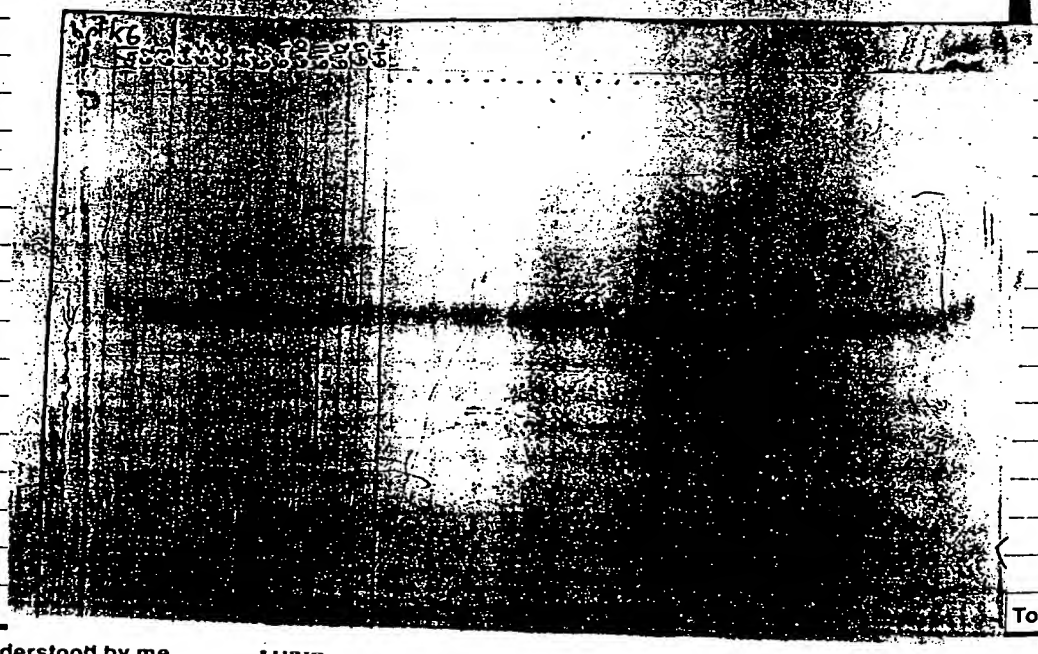
Projec[illegible]o._____

Book No. 19211 TITLE TESTING & HPTKG - SERA

From Page No.___



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Witnessed & Understood by me,

Date

Recorded by

30th Nov 93

TITLE TESTING of HPTK-6 SERA.

Project No.
Book No. 19211

From Page No.

Two batches of test serum obtained together with prebleed controls

Rabbit #	PRE-BLEED DATE	BLEED AFTER SECOND BOOST
18243 - 71A	8/11/93	9/30/93
18243 - 71B	8/11/93	9/30/93

Used strips as follows

Strip #	SERUM	DIL'N	VOLUME
1	71A (Prebleed)	1/100	100λ
2	71A	1/100	100λ
3	71A	1/1000	10λ
4	NONE - SECONDARY ALONE.		
5	71B (Prebleed)	1/100	100λ
6	71B	1/100	100λ
7	71B	1/500	20λ
8	71B	1/1000	10λ

Developed using 2°-anti-rabbit - Phosphatase (Boehringer) with BCIP + NBT.

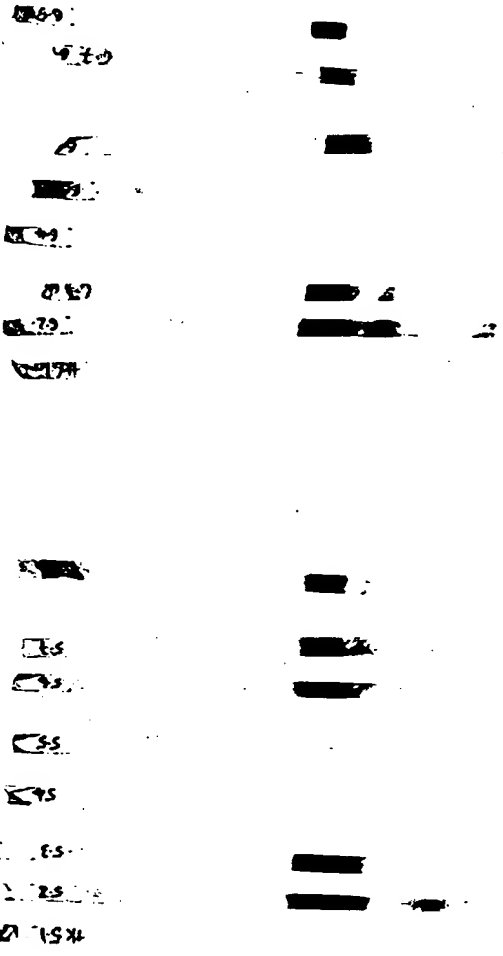
To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by P. Bell	30th Nov. 93

Project N

Book No. 19211 TITLE TESTING & HPTK6 SCRA

From Page No.__



FRIDAY 3rd Nov 93

To Page No.____

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by [redacted]	30th Nov 93

TITLE TESTING OF HPTK6 SERA

Proj No.

Book No. 9211

From Page No.

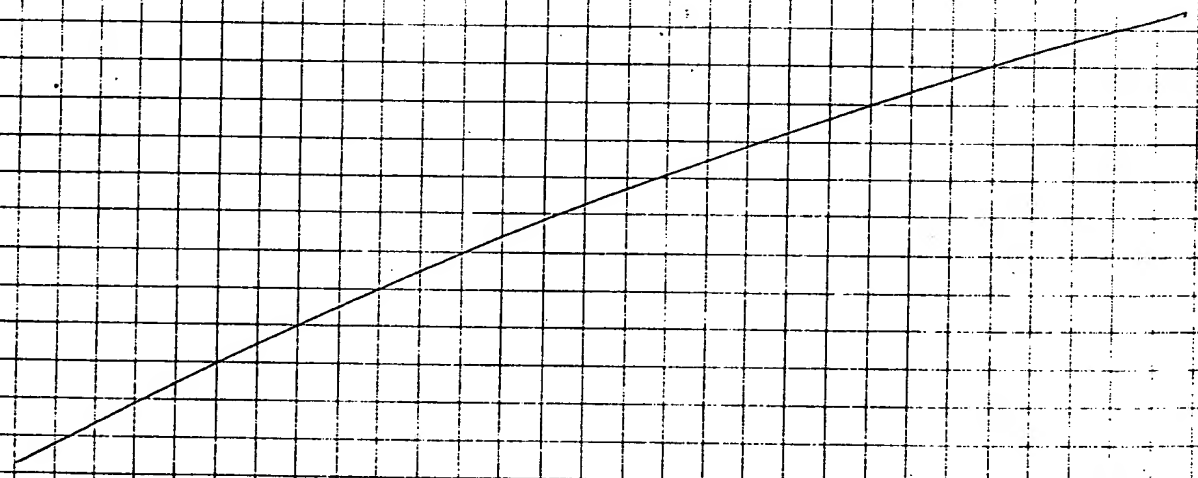
RESULTS & CONCLUSIONS

There appears to be a good differential reaction with the 71-B serum when used at especially 1/1000 dilution.

- There is a much stronger reaction against hptk6 than hptk5 on str.jx #8.

This can also be seen to a lesser extent with the lower dilutions of 71B, and perhaps with the 71A serum also.

The prebleeds and the no-primary controls are clean



To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by [illegible]	30 Nov. 95

6

Project No.

Book No. 19211 TITLE SCREENING CHO - FULL LENGTH CLONES

From Page No.

Aim

Use the 7H-B antiserum to screen the 20 clones that Will Barron has generated that potentially express full-length HPTK-6.

Plates of 20 CHO cell lines were used; also used untransfected CHO cells

90mm Plates were confluent and grown in F12/DMEM -GHT.

Removed medium from cells, rinsed in PBS, then added 10mls PBS + 5mM EDTA

- left at 37°C for 20-30 mins

Cells were transferred to 15ml Falcon tubes, washed 2x with PBS & then stored frozen at -20°C.

(21st Nov 93)

- The thawed cells were lysed by resuspension into 450µl of RIPA buffer + PMSF + Aprotinin, left on ice for 15mins prior to spinning 2-3mins at 2000 rpm in the microfuge.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by [illegible]	30th Nov 93

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400µl of the supernatant was transferred to a new tube and 200µl of 3x Sample Buffer (+β-ME) was added.

→ Samples were heated at 95°C for 10mins &

80λ aliquot loaded onto 8% SDS-PAGE gels

GELS 1+2 ;

M_R 1 → 15

GEL 3

M_R 16, 17, 18, 19, 20, CHO [ ] M_r, 16 → 20, CHO.

Ran slowly o/n.

(22nd Nov '93)

Blotted gels to N/C filters as normal,

Ponceau stained – photocopies shown overleaf, (Page 8)

To Page No.____

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	23rd Nov 93

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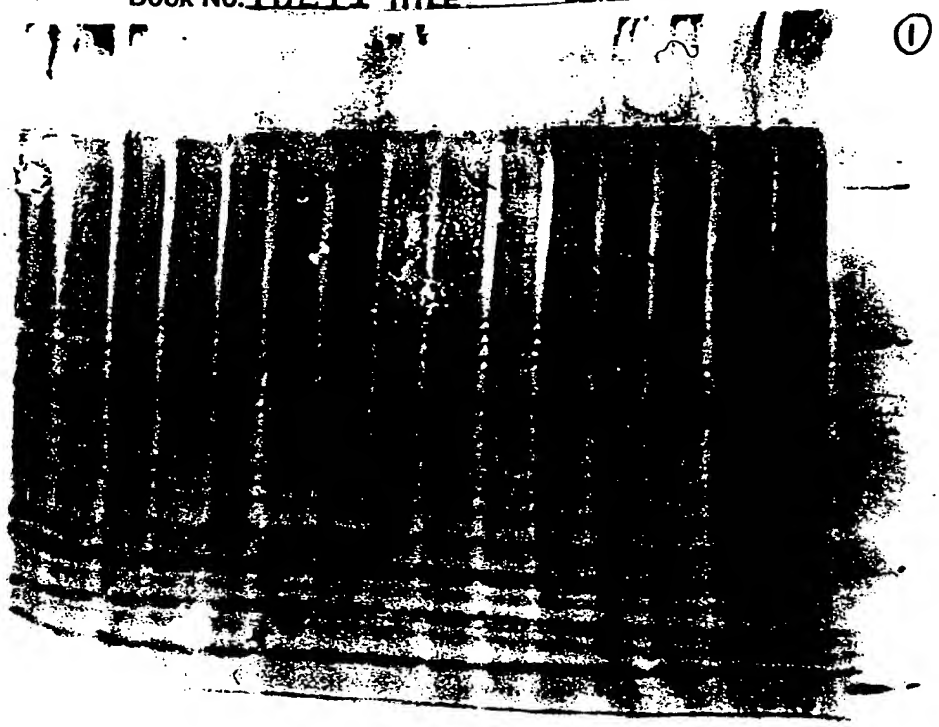
Project I. _____

Book No. 19211 TITLE SCREENING [illegible]

From Page No.



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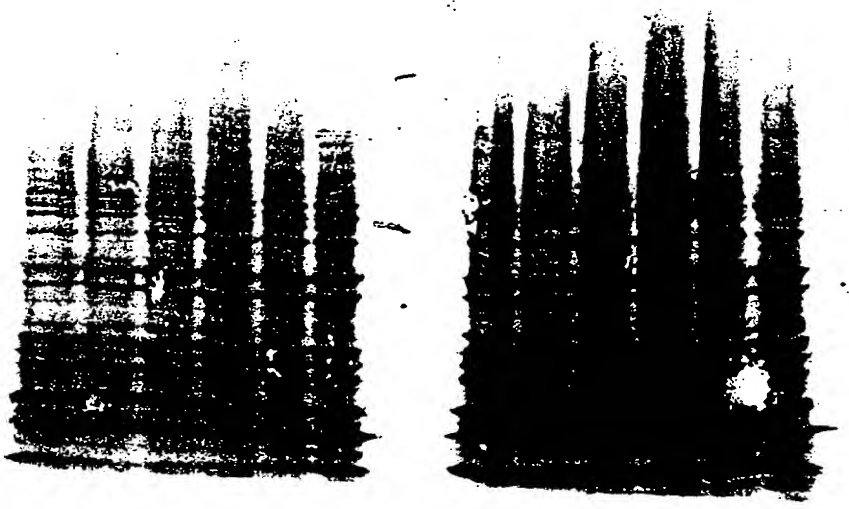


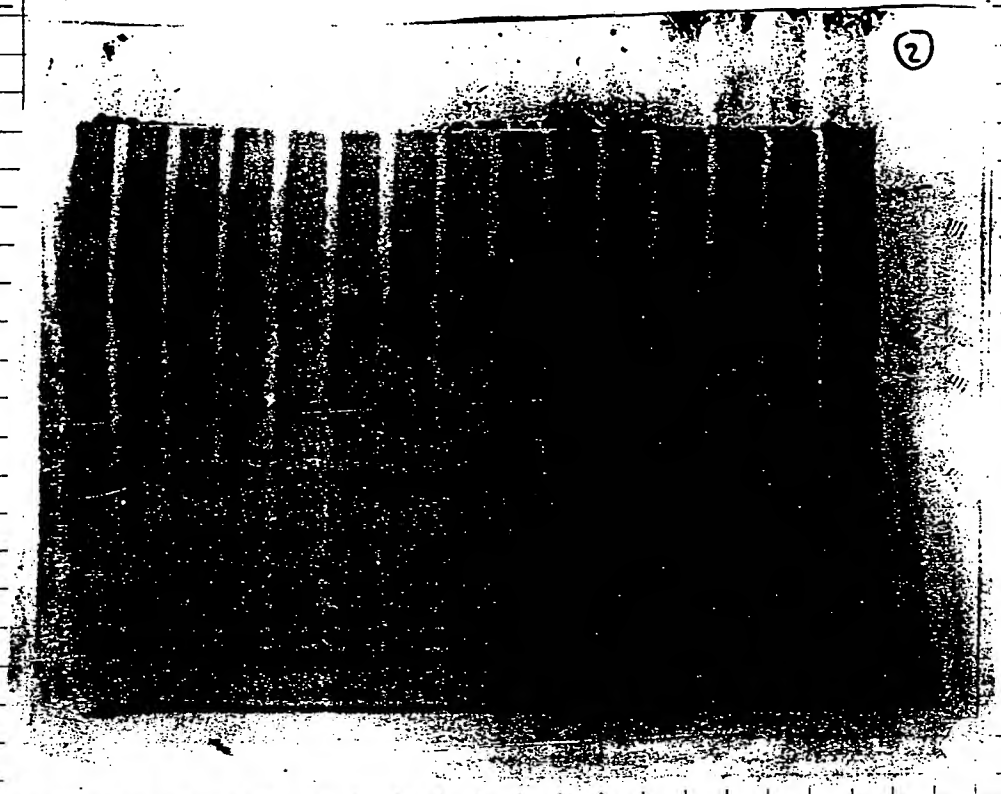
Page No.____

Witnessed & understood by me,

Recorded by [illegible] 30th Nov 93

TITLE SCREENING FULL LENGTH EXPRESSION. Proje[illegible]o. Book No. 9211

From Page No.



Gel 3 split with 2 equivalents - gels 3+4.

GEL 1 + 3 probed with 1/1000 anti-hptk-6 71B as used previously ;

Gels 2 + 4 Probed with UBI monoclonal anti - (P) - Tyr.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date 30th Nov '93
		Recorded by G P. Thiru	

10 Project I. _____ Book No. 19211 TITLE _____

From Page No. __

Developed with 2° - anti rabbit or anti-mouse (P-Tyr) - phosphotyrosine linked & BCIP + NBT

Alkaline Phosphatase Reaction :

- Wash Blot with Alk Phosph. Buffer 5 mins.

- Make up Reaction Soln : 20 mls Alk Phosph Buff.
132 µl Nitro blue tetrazolium
66 µl BCIP

RESULTS & CONCLUSIONS

Blots are shown on Page 11 for α hptk-6
Page 12 for α-(P)-Tyr.

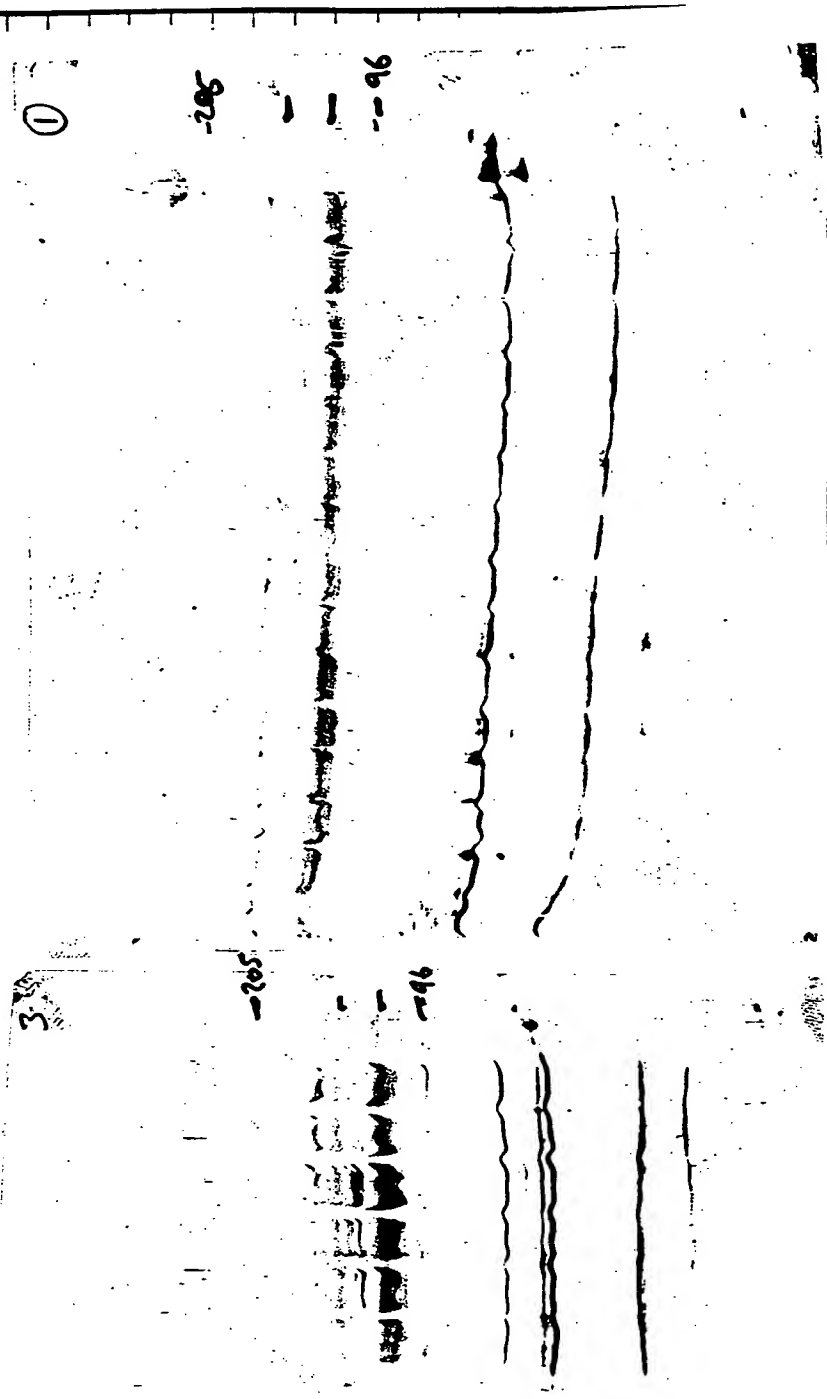
Sample # 18 appears to have a band of the approx. right Mr coming up - this may also be present to a lesser extent in #19 & #20 too : + much lower in #4.

In addition a band of much higher than expected Mr appears to be well expressed in #16 and to a lesser extent in #1, #17 #18.

To Page No. __

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by N. P. Hunt	30th Nov 93

From Page No.__



To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	30th Nov 93

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(2)

ANTI-(D)-IXR

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by [illegible]	30th Nov. 93

From Page No.

The anti-phosphotyrosine blot shows no band of the right mol wt for hptk-6, suggesting that the receptor is not phosphorylated under the conditions - Note I did not add phosphatase inhibitors to the cells when I lysed them and this may be important! However, there is a distinct band of approx 80 kDa coming up in most of the transfected samples c.f. CHO cells alone. Moreover this band appears strongest in sample #18.

This may be related to hptk-6 expression - perhaps a target for the kinase

There results should tie in with whatever W.H. Baron finds out from a FACS analysis of these 70 cell lines using this antiserum.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	30th Nov. 93

14 Project No. Book No. 19211 TITLE TESTING FURTHER α HPTK6 BLEEDS.

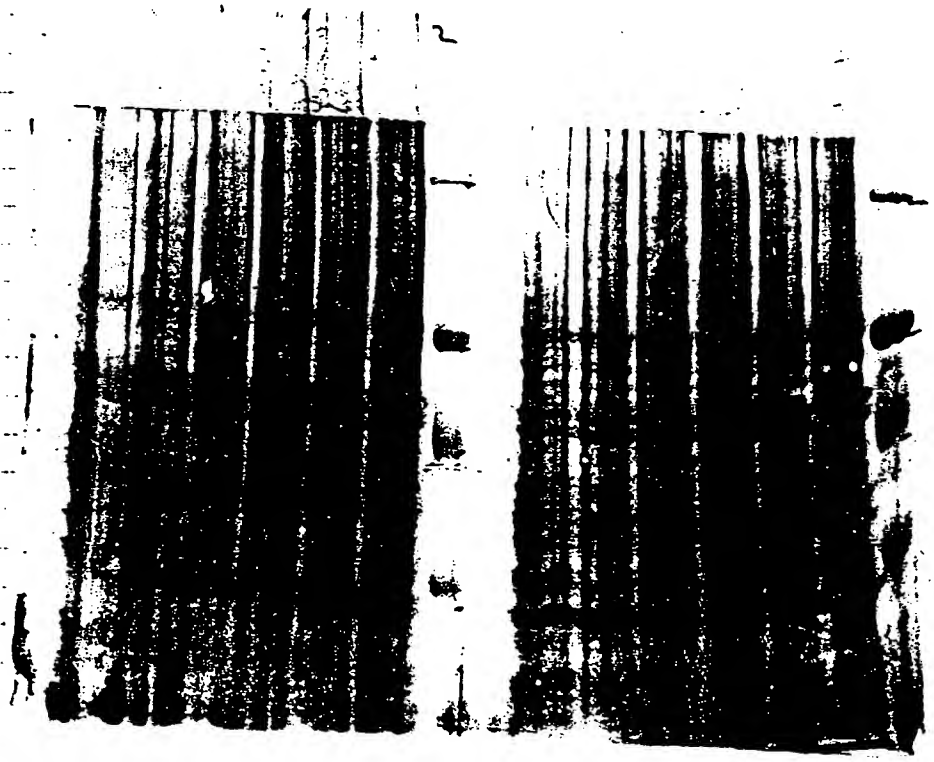
From Page No.

Monday 6th Dec 93.

Ran 60µl aliquots of samples prepared on Nov 21st 93 on 0.8% gel.

Ran gel fast (~60mA)

Blotted to NK Filter ; Ponceau Stained + photocopied.



Gel overloaded + ran too fast!!

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by [signature]	Dec 7th 93

TITLE TESTING FURTHER BLEEDS

Project No. Book No. 1921i

From Page No.

Tuesday 7th Dec. 93

Obtained 2 new bleeds from rabbit 71-B ; bled from 10/28/93 & 11/18/93.

Used 1/500 dilutions of these to decorate the blot :

Panel (1) : 10/28/93
Panel (2) : 11/18/93.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by KP Bhv	7th Dec '93

From Page No.

RESULTS & CONCLUSIONS

Plot too overloaded + messy to make any definite conclusion about this

- Need to repeat using perhaps 30λ chords of the sample

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	7 Dec 93

TITLE TESTING HMTK6 BLEEDS AGAIN.

From Page No.

7th Dec. 93

Paired 2 New 8% gels.

loaded with samples as previous

ie Mrs 16, 17, 18, 19 20 CHO, Mrs 16, 17, 18, 19 20 CHO

Ran gels slowly o/n.

8th Dec 93

Transferred to N/C filters

Pancew stained - Copies attached Page 18,

Blotted with

PANEL	PROBE
1	71B
2	71B
3	71-B - Pre bleed.
4	No Primary Ab.

- 2hrs RT; Wash; then Probed all with 1/1000 of Anti-Rabbit - Alk Phosph. conjugate

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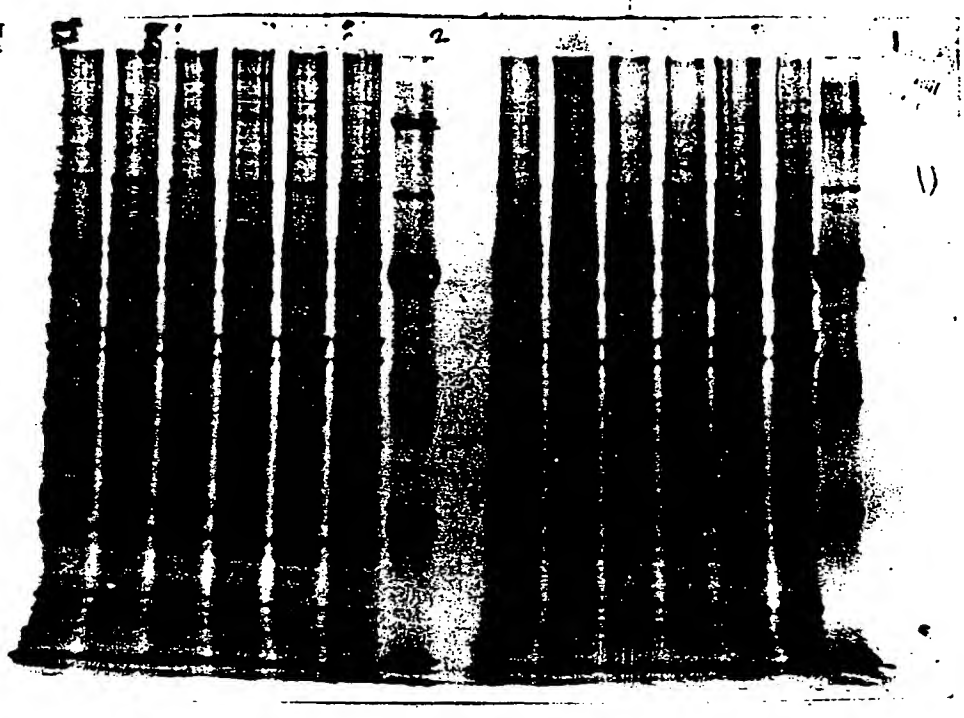
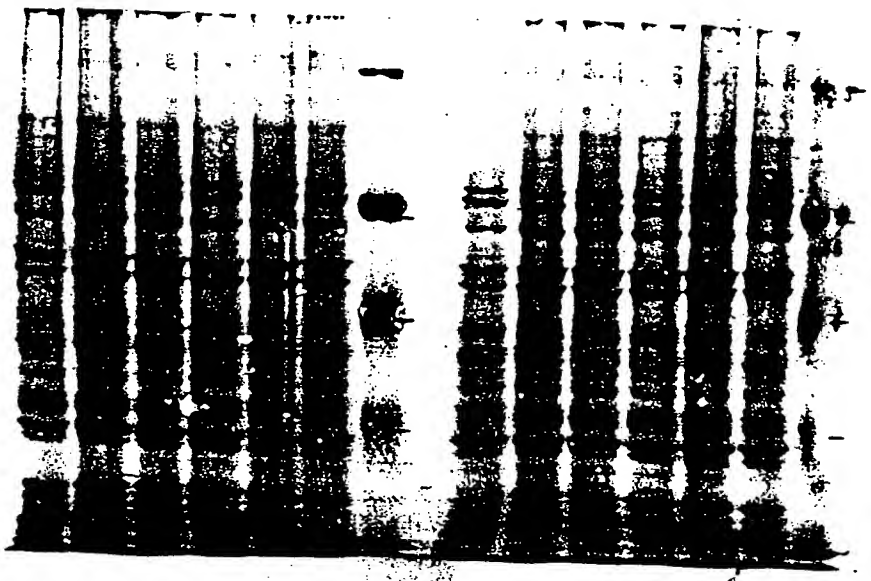
Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	9th Dec 93

From Page No.





Page No.

Witnessed & Understood by me,

Date

Invented by

Recorded by

9th Dec 93

From Page No.

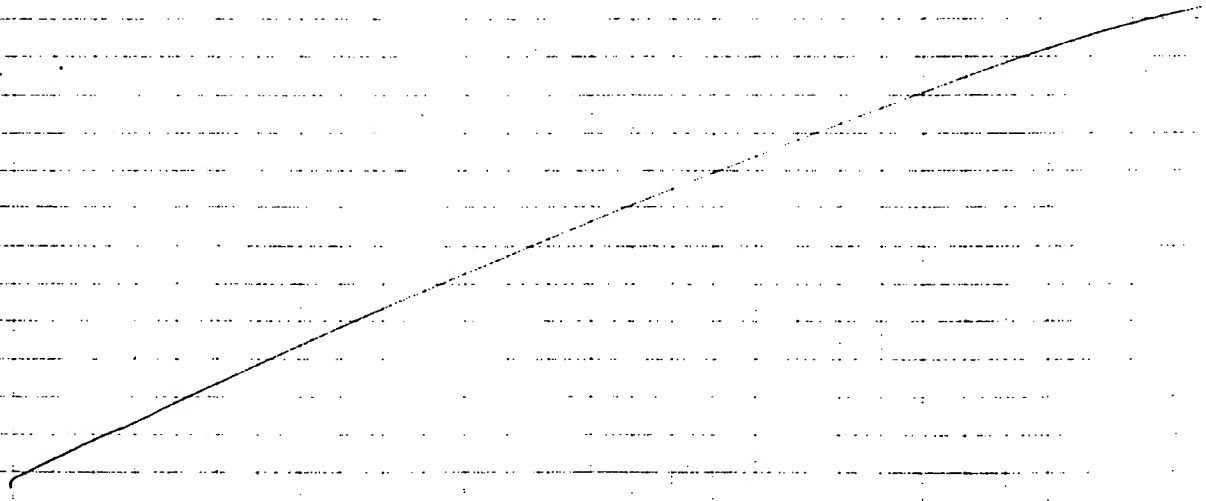
Developed with BCIP/NBT as usual.

;- Western attached Pge 20

RESULTS & CONCLUSIONS

- Gels are much cleaner than before, although I do not see any major bands that are obvious in the transfected cell lines cf. CHO untransfected.

There are a couple of minor bands at about the approx right Mr



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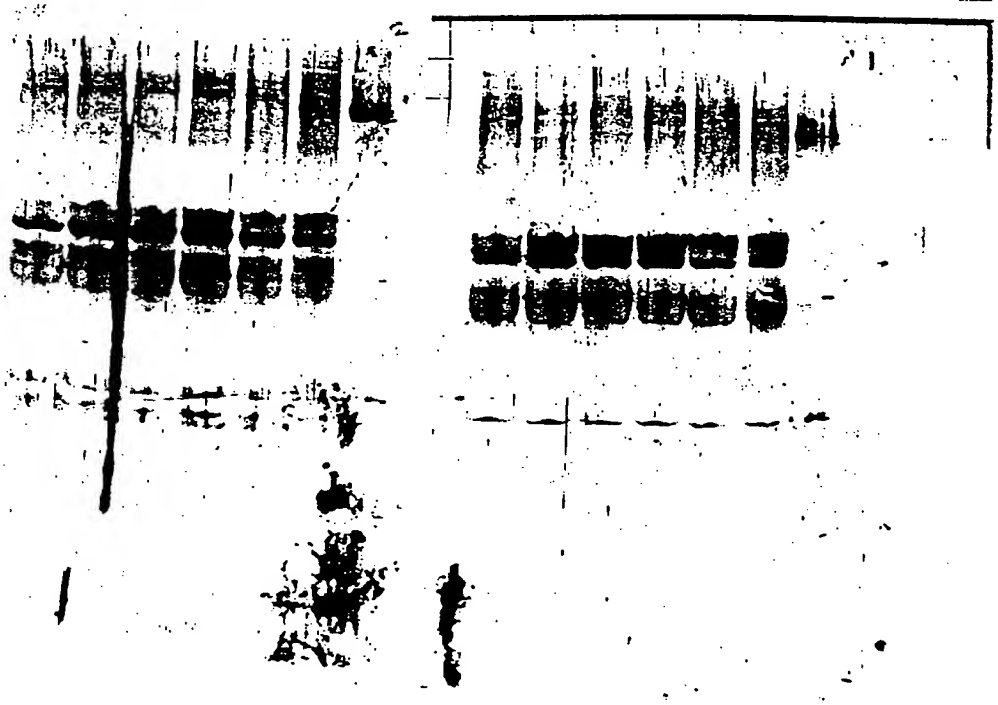
		Recorded by	9th Dec 97

From Page No.



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Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	4th Dec 93

From Page No.__

9th Dec 93

- Cells in 6 well plate ~~dishe~~ plates

Well 1 : FL-16

2 FL-17

3 FL-18

4 FL-19

5 FL-20

6 CHO

Cells were passed ~20 hrs before by Will Benson

- Inoculated with 250-500 $\times 10^3$ cells ; grown o/n in DMEM + serum

: Microscopic examination suggests about 50% confluency

- Removed media by suction

- washed with 3mls pre-warmed serum free DMEM + HEPES

- Sucked off wash

Added 1ml of serum free DMEM + HEPES.

Incubated at 37°C for 60 mins

To Page No.____

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by 10th Dec 93	

22 Project No._____ Book No. 19211 TITLE (P)-74K AFTER STIMULATION.

From Page No.__

- Added Abs = 20x of each as shown below
- This represents a 1/50 dilution of the serum.

PLATE 1	- no additions	(unstimulated)
PLATE 2	- 71-B-①	5min stimulatn
PLATE 3	71-B-①	30 min stimulatn
PLATE 4	71-B-②	15min
PLATE 5	71-B-2	30min
PLATE 6	71-B- Prelmmune	5 min.
PLATE 7	71-B- Prelmmune	30mins

① = 10/28/93 bleed

② = 11/18/93 bleed

Prelmmune = 8/11/93 bleed.

- Removed sups after the shown times & washed cells 1x with PBS.
  - ⇒ Removed PBS
  - Added 300µl of 1x SB.

To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	10th Dec 93

TITLE (P-TYR -WESTERNS, AFTER STIMULATION. Proj No. Book No. 19211

From Page No.

Transferred to eppendorf tubes. Heated 95°C for 5 min.

Loaded ½ vol (30µl) to 8% PAGE-SDS.

Tubes labelled as follows:

Plate 1 → 1-1 ← Well 1 2-1 . 3-1 ... etc

1-2
1-3
1-4
1-5
1-6

Gels loaded as follows:

GEL 1 FL-16: Mr 1-1 2-1 3-1, 4-1, 5-1, 6-1, 7-1 Mr FL-17: 1-2, 2-2, 3-2, 4-2, 5-2, 6-2, 7-2

GEL 2: Mr FL18: 1-3, 2-3, 3-3, 4-3, 5-3, 6-3, 7-3, Mr FL-19: 1-4, 2-4, 3-4, 4-4, 5-4, 6-4, 7-4

GEL 3 Mr FL-20: 1-5, 2-5, 3-5, 4-5, 5-5, 6-5, 7-5 Mr CHO: 1-6, 2-6, 3-6, 4-6, 5-6, 6-6, 7-6

Ran gels slowly o/n.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by:	10. Dec 93.

From Page No.__

10th Dec 93

Blotted gels to N/C filters.

Very little protein on the filters ; transfer worked fine because the markers are ok.

- Eg Pancreas of gel #3.

Decorated gel with UBI's anti-Ⓟ-tyr Monoclonal Ab.

Blocked filters in PBS/ 5% BSA / 0.05% Tween-20.

:- 2-3 hrs Room Temp.

; Wash 3x PBS

To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	10. Dec 93

TITLE _____ Proj. No. Book No. 19211

From Page No.___

- 2° Ab was Anti-mouse - Phosphatase (Boehringer)
- 2hrs in PBS / 5% BSA / Tween-20
- washed 3x PBS
- 1x Alk Phosph. buffer
  - 100mM Tris-Cl pH 9-5
  - 100mM NaCl
  - 5mM $MgCl_2$
- Developed with NBT / BCIP.
- No immediate staining was apparent, so let the staining proceed o/n. Stained gels attached Pages 26+27

RESULTS & CONCLUSIONS

Not enough protein loaded on the gels - need to repeat the ~~of~~ gels + western with these samples.

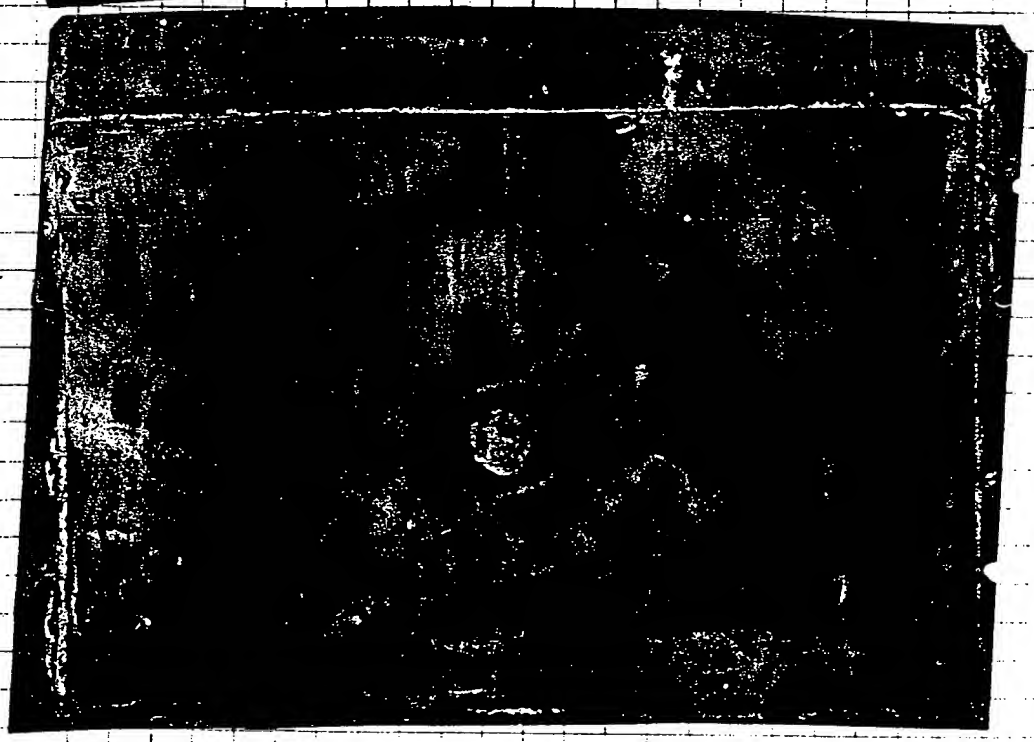
To Page No.___

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	13th Dec 93

Project N.

26 Book No. 19211 TITLE

From Page No.



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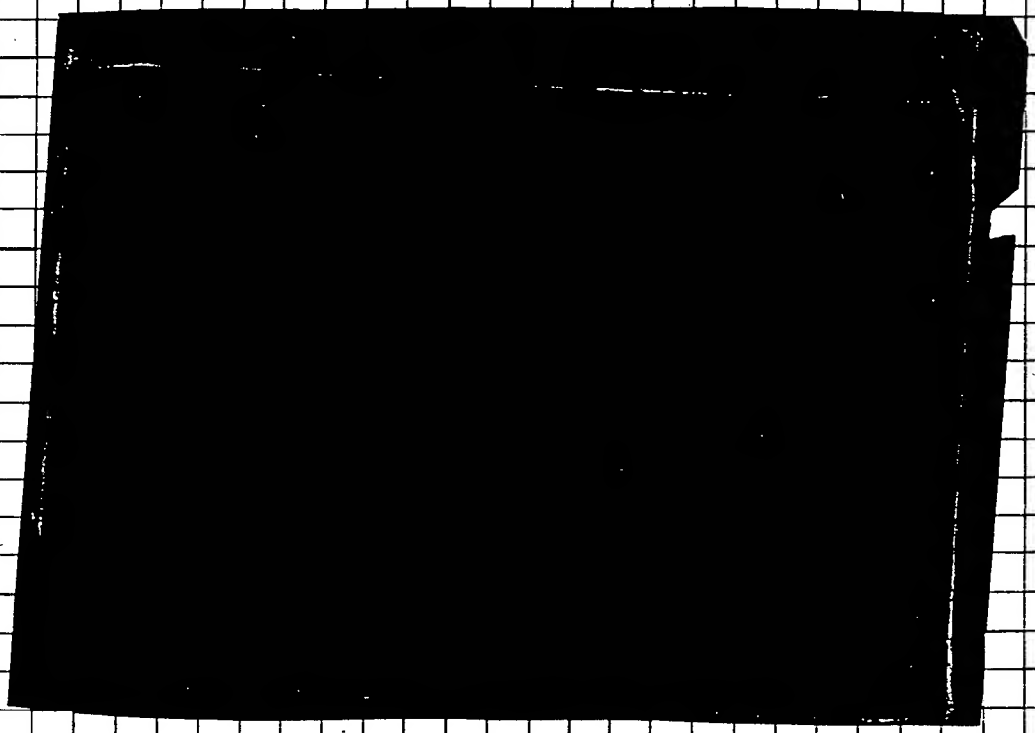
Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by [illegible]	13 Dec 97

TITLE _____ Project No. Book No. 19211

From Page No.



REPEAT OF GELS

13TH Dec 93

Loaded 10µl of redried sample (left top open when heating to 95°C for 10 mins) on to new 8% gels

- Ran gels at 30 mA for ~5 hrs

Blotted to N/C filters for ~2 hrs.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by JCP Tyler	13 Dec 93

Project No

28

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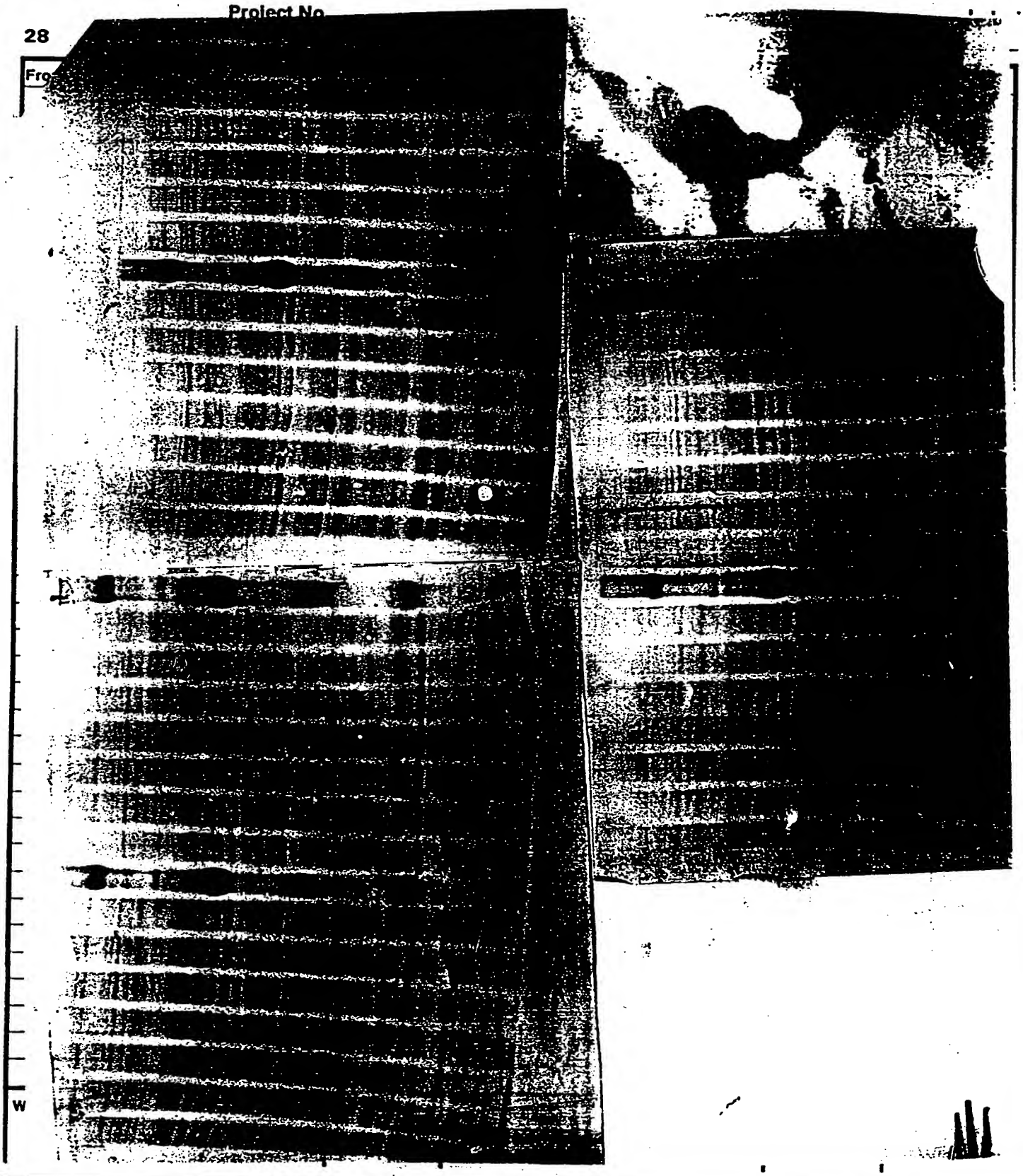

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TITLE (P)-TYR AFTER STIMULATION. Project No. Book No. 19211

From Page No.

14th Dec. 93

Decorated the Blots as previously described - using the MAb (stored at -20°C)

- Developed using NBT/BCIP.

- Blots attached Pages 30 + 31

RESULTS & CONCLUSIONS

No specific bands are obvious after stimulation with the polyclonal Ab serum ;-

There is a prominent band in CHO cells alone, at around this size which has (P)-Tyr ; this may obscure any bands that are coming up with stimulation.

Alternately there may be no stimulation of kinase activity with these serum / bleeds ; new bleeds should be tried.

Also keep in mind a metabolic (S^{35}) labelling followed by anti-(P)-Tyr immuno-ppt.

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Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	14th Dec 93.

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Project No.

Book No. 19211 TITLE

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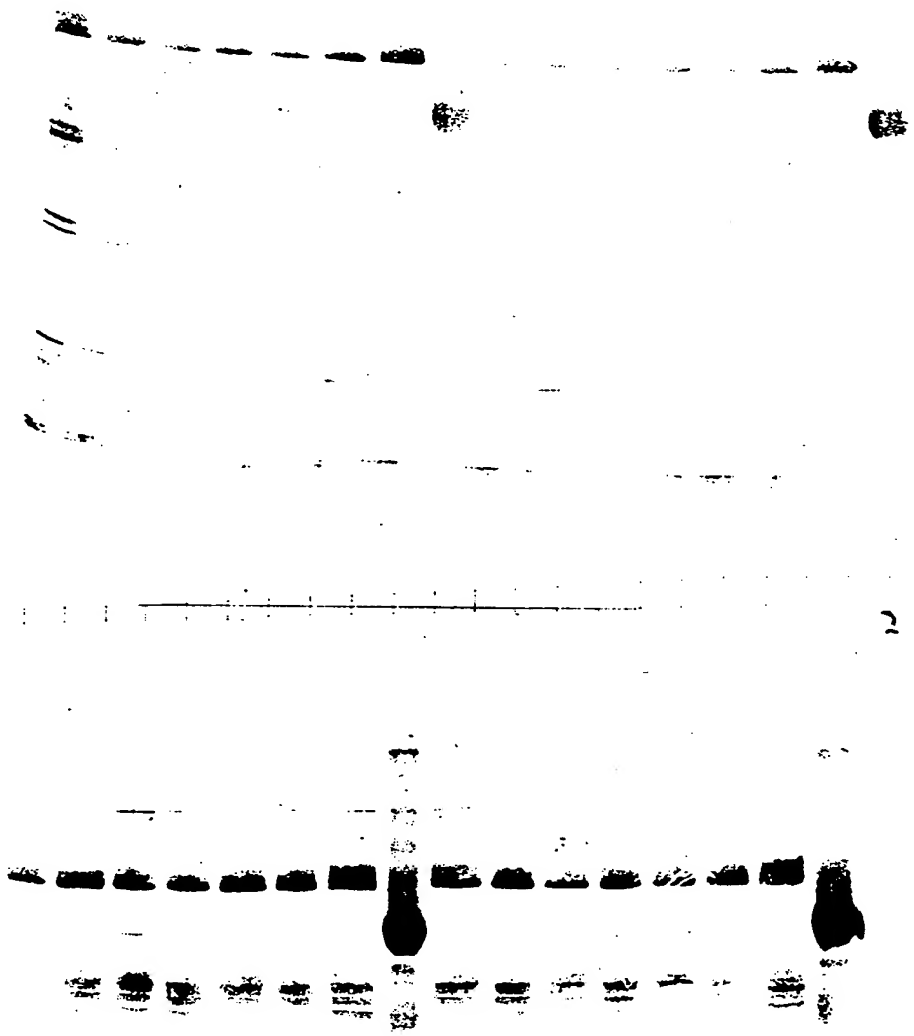

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Witnessed & Understoc	Date	Invented by	Date
		Recorded by	15th Dec 93

Project No.
TITLE _____ Book No. 19211

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3

15th Dec 97.

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Witnessed & Understood by me,	Date	Invented by	Date 15th Dec 97
		Recorded by	